

On the Structure of the New
Amino Acid A, 2-Methylenecyclo-
heptene-1,3-diglycine, Isolated
from the Mushroom

Lactarius helvus

ERKKI HONKANEN, TAUNO MOISIO,
ARTTURI I. VIRTANEN

Laboratory of the Foundation for Chemical
Research, Biochemical Institute,
Helsinki, Finland
and ATTILIO MELERA

Varian A.G., Research Laboratory,
Zürich, Switzerland

In an earlier paper by Casimir and Virtanen¹ the occurrence of four new amino acids (A, B, C, and D) in the mushroom *Lactarius helvus* was described. All these amino acids give on the paper chromatogram with ninhydrin characteristic yellow or yellow-brown spots which after a while turn violet. Komamine and Virtanen,² using the method of Linko,³ showed that the amino acid A contained two α -amino and α -carboxyl groups. On catalytic hydrogenation two moles of hydrogen, or on bromination two moles of bromine, were consumed per mole of the amino acid, showing the presence of two double bonds. The double bonds were not conjugated. Elemental analysis of the compound A gave an empirical formula of $C_{11}H_{18}N_2O_4$.²

In continued investigations with a larger amount of material it was observed that the so-called amino acid A was a mixture

of three, perhaps isomeric compounds (A_1 , A_2 , and A_3). By crystallization of this mixture several times from water, the amino acid A_1 most difficultly soluble in cold water was obtained in pure form. Its IR-spectrum showed it to be identical with the amino acid A prepared by Komamine and Virtanen.² On catalytic hydrogenation, amino acid A_1 gave two dihydrogenation products, indicating that a new asymmetric carbon atom was formed in the molecule and that the carbon chain must be branched at the position of the double bond. The NMR spectrum of the amino acid A_1 showed that it contained no methyl group. Two ring olefinic and two terminal olefinic protons were, however, evident. After catalytic hydrogenation a well defined signal of a secondary methyl

group $H-\overset{|}{\underset{|}{C}}-CH_3$ was found. The amino

acid A_1 must therefore contain a $>C=CH_2$ group, from which the new asymmetric carbon atom is formed on hydrogenation.

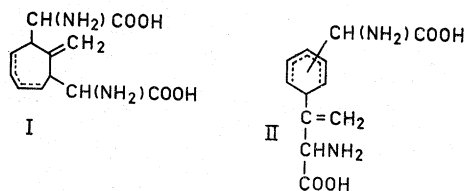
By oxidation of the amino acid A_1 with ninhydrin followed by steam distillation only traces of a volatile compound were obtained. The mass spectrum of this compound was typical of a dialdehyde having a molecular weight of 164, indicating the formula $C_{10}H_{12}O_2$ for this dialdehyde. Its 2,4-dinitrophenyl-hydrazone showed an absorption maximum at 355 m μ , which is characteristic of aldehydes having no α,β -double bonds. Therefore the double bonds in the dialdehyde molecule are not conjugated with the carbonyl groups.

By ninhydrin oxidation of the hydrogenated amino acid the corresponding saturated dialdehyde (mol. wt. 168, $C_{10}H_{16}O_2$)

was obtained in much better yield. This saturated dialdehyde gave with silver oxide a dicarboxylic acid $C_{10}H_{16}O_4$ (Found: C 60.34; H 8.10. Calc. for $C_{10}H_{16}O_4$: C 59.98; H 8.05.), from which the corresponding dimethyl ester (mol.wt. 228. Found: C 63.35; H 8.73. Calc. for $C_{12}H_{20}O_4$: C 63.13; H 8.83.) was obtained with diazomethane.

On the basis of these results the true formula of the original amino acid A_1 must be $C_{12}H_{18}N_2O_4$. In addition the molecule must contain a ring, since only two double bonds are present.

The nuclear magnetic resonance studies of the original amino acid A_1 , its diethyl ester and that of the dimethyl ester $C_{12}H_{20}O_4$ lead to the following possibilities (I and II) for the structure of the amino acid A_1 :



The structure II would give on ninhydrin oxidation an α,β -unsaturated aldehyde. This is, however, not in agreement with the experimental results. This being the case the structure I, 2-methylenecyclohept-(4,5 or 6)-ene-1,3-diglycine, corresponds best with the before-mentioned facts. The position of the second double bond remains still in doubt.

Full details of these investigations will be published later.

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